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Activity recording

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Multicolor three-photon fluorescence imaging with single-wavelength excitation deep in mouse brain

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Detailed protocol

Activity recording

Animal preparation

For functional imaging in the mouse cortex, we used GCaMP6s-labeled transgenic mice with high labeling density throughout the cortex. For Fig. 3, we crossed Cre-lox transgenic mice (Slc17a7-IRES2-Cre (B6;129S-Slc17a7$^{tm1.1(cre)Hze}$/J, JAX stock number 023527 (*1*)) and Ai162D reporter mice (JAX stock number 031562 (*2*)) to drive GCaMP6s expression in excitatory neurons throughout the cortical areas. For Fig. 3, a 9-week old male mouse (25 g) was used. For Fig. S9, a GCaMP6s transgenic mouse (CamKII-tTA/tetO-GCaMP6s, female, 8 weeks old, 18 g) was used. Craniotomy was performed prior to the mouse brain imaging. Procedure for mouse brain craniotomy followed closely to what was described previously (*3*). Body temperature was kept at 37.5 °C with a feedback-controlled blanket (Harvard Apparatus), and eye ointment was applied. Prior to surgery, glycopyrrolate (0.01 mg/kg body weight), dexamethasone (0.2 mg/kg body weight), and ketoprofen (5 mg/kg body weight) were injected intramuscularly. The craniotomy was centered at ~2.5 mm lateral and ~2 mm caudal from the bregma point over the somatosensory cortex. The window was further stabilized with dental cement covering the skull and the 5-mm diameter circular coverslip (#1 thickness, Electron Microscopy Sciences). A homemade metal holder was attached to the skull with dental cement, which was used to hold the mouse brain during activity recording. For Fig. 3, 200 µL of 25 mg/ml Texas Red (dextran conjugate, 70,000 MW, Thermo Fisher Scientific) was injected retro-orbitally prior to imaging. For SR 101 imaging in Fig. S9, SR 101 solution in phosphate buffered saline with concentration of 300 µM was topically applied on top of the dura for 10 min after opening the skull (*4*, *5*), before attaching the cranial window. During all surgeries, the mice were anesthetized with 1.0 to 1.5% isoflurane in oxygen.

Activity recording experiment

A non-collinear optical parametric amplifier (Spirit NOPA, Spectra Physics) pumped by a hybrid fiber-solid-state laser (Spirit 1030-70, Spectra Physics) was used as the excitation source. The excitation wavelength was 1340 nm, and the laser repetition rate was set at 2 MHz. The multiphoton images were acquired with a commercially available microscope (Bergamo II, Thorlabs) that has four detection channels with GaAsP PMTs (PMT2101, Thorlabs). A high NA objective lens (XLPLN25XWMP2, Olympus, NA = 1.05) was used with D_2O as the immersion medium. The diameters of the beams at the objective lens back aperture (aperture size: ~15 mm) were ~13 mm ($1/e^2$). The average power under the objective lens was 56 mW and 70 mW for Fig. 3E,F and Fig. S9B,C. For Fig. 3E,F and Fig. S9B,C, the activity recording was performed at 750 µm and 762 µm beneath the dura, respectively, with a field-of-view of 270 × 270 µm (256 × 256 pixels). The frame rate was 8.3 Hz, with a pixel dwell time of 0.51 µs. The signal was epi-collected through the objective lens and then reflected by a 705 nm long-pass dichroic beam splitter. The signals were separated into four detection channels by the 562-nm, 488- and 635-nm (FF562-Di03, Di02-R488 and Di02-R635, Semrock) dichroic beam splitters, and the three short-wavelength channels were used for the activity recording. Three band pass filters were used for the three channels: centered at 447 nm (FF02-447/60, Semrock), 525 nm (FF03-525/50, Semrock), 617 nm (FF02-617/73, Semrock). The 447-nm channel was used to detect the third-harmonic generation (THG) of 1340-nm excitation; the 525-nm channel was used to detect fluorescence from GCaMP6s; and the 617-nm channel was used to detect fluorescence from Texas Red and SR 101. The Ca^{2+} recording was performed for 585 s and 193 s for Fig. 3E,F and Fig. S9B,C, respectively.

Analysis of the activity recording data

For Ca^{2+} activity recording, mechanical drift and mouse motion in the horizontal plane were corrected with the Template-Matching plug-in in ImageJ by using the fluorescence signals from Texas Red (for Fig. 3E,F) or SR 101 (for Fig. S9B,C) as a template for each frame. Regions of interest corresponding to the neurons were visually identified and selected. Baseline of the traces (F_0) is defined as approximately the lowest 20% of each trace during the whole recording time. Fluorescence traces (F) are normalized according to the formula $(F - F_0)/F_0$. For the activity videos shown in Movies S2 and S3, 16-frame rolling average, Kalman filtering (gain: 0.9), and gamma correction with a correction value of 0.8 were applied for the purpose of visualization.

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